

CLAIMS

What is claimed is:

1. An isolated nucleotide fragment comprising:
 - (a) a nucleic acid sequence encoding a ryanodine receptor having an amino acid sequence identity of at least 80% when compared to a polypeptide selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 128, 130, 144, and 146; or
 - (b) the complement of (a).
2. The isolated nucleic acid fragment of claim 1 wherein the sequence identity is at least 85%.
3. The isolated nucleic acid fragment of claim 1 wherein the sequence identity is at least 90%.
4. The isolated nucleic acid fragment of claim 1 wherein the sequence identity is at least 95%.
5. The isolated nucleic acid fragment of Claim 1 wherein the amino acid sequence of the polypeptide comprises the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 128, 130, 144, or 146.
6. An isolated nucleic acid fragment comprises a nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 127, 129, 143, or 145.
7. A recombinant construct comprising the isolated nucleic acid fragment of claim 1 or 5 operably linked to at least one regulatory sequence.
8. A transformed host cell comprising the recombinant construct of Claim 7.
9. The host cell of claim 8 wherein said cell is selected from the group consisting of E.coli, yeast, Sf9, Sf21,S2, Xenopus oocytes, HEK-293 and CHO.
10. A method to isolate nucleic acid fragments encoding ryanodine receptors and related polypeptides, comprising:
 - (a) comparing SEQ ID NO:2, 4, 6, 8, 10, 128, 130, 144 and 146, and other ion channel and receptor polypeptide sequences;
 - (b) identifying the conserved sequences of 4 or more amino acids obtained in step a;
 - (c) designing degenerate oligomers based on the conserved sequences identified in step b; and
 - (d) using the degenerate oligomers of step c to isolate sequences encoding polypeptides having ryanodine receptor activity by sequence dependent protocols.

11. An isolated polypeptide having ryanodine receptor activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 128, 130, 144, or 146, have at least 80% identity.

12. The polypeptide of Claim 11, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 128, 130, 144, or 146, have at least 85% identity.

13. The polypeptide of Claim 11, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 128, 130, 144, or 146, have at least 90% identity.

14. The polypeptide of Claim 11, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 128, 130, 144, or 146, have at least 95% identity.

15. The polypeptide of Claim 11, wherein the amino acid sequence of the polypeptide comprises the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 128, 130, 144, or 146.

16. A method for evaluating at least one compound for its ability to modulate calcium homeostasis, the method comprising the steps of:

(a) transforming a host cell with the recombinant construct of Claim 7;
(b) growing the transformed host cell under conditions that are suitable for expression of the recombinant construct wherein expression of the recombinant construct results in altered calcium homeostasis;
(c) treating the transformed host cell of step (a) with a compound to be tested; and

(d) determining changes in intracellular calcium homeostasis by the test compound in order to select compounds with potential for altering calcium release.

17. A method for evaluating at least one compound which modulates ryanodine receptor activity, the method comprising the steps of:

(a) contacting at least one compound with a polypeptide encoded by an isolated nucleic acid fragment of claim 1; and

(b) evaluating the ryanodine receptor activity of the polypeptide of (a) after said polypeptide has been contacted with the compound or compounds.

18. The method of claim 16 or 17 wherein the method is a ligand binding assay.

19. The method of claim 16 wherein the method is based on assessing functional activity by detecting the effect of a compound on the functional activity of the transformed host cell.

20. The method of claim 19 wherein the polypeptide is contacted with more than one compound.

21. An isolated nucleic acid fragment encoding an insect ion channel comprising at least two polypeptide sequences set forth in any of SEQ ID NOs:63-119 provided that said polypeptide sequences do not comprise any of SEQ ID NOs: 56, 120-126.

5 22. A method for identifying a nucleic acid sequence encoding an insect ion channel comprising:

a) obtaining an isolated nucleic acid sequence encoding a first polypeptide having at least 100 amino acids;

10 b) comparing the first polypeptide sequence with a comparative polypeptide sequence selected from the group consisting of SEQ ID NOs:63-119 to identify a region between the first polypeptide and the comparative polypeptide having 100% sequence identity wherein said region is as long as the comparative polypeptide; and

15 c) repeating step (b) with a different comparative polypeptide sequence, wherein said different comparative polypeptide sequence is selected from the group consisting of SEQ ID NOs:63-119 until a second region having 100% sequence identity is found, wherein said second region is as long as the different comparative polypeptide.

20 23. The method of claim 22 wherein the first polypeptide has a length from 100 to 6,000 amino acids.

24. A method for expressing an isolated nucleic acid fragment encoding a toxic insect ion channel which comprises:

25 a) transforming a host with a recombinant construct comprising in the 5' to 3' direction a promoter operably linked to the toxic insect ion channel nucleic acid wherein the promoter comprises a transcription termination nucleic acid fragment situated between said promoter and the isolated nucleic acid fragment encoding the toxic insect ion channel, and

30 further wherein the transcription termination nucleic acid fragment is flanked on each end by at least one nucleic acid sequence consisting essentially of excisable sequences; and

b) growing the transformed host under conditions that are suitable for the expression of the recombinant construct.

25. A method for expressing an isolated nucleic acid fragment encoding a toxic insect ion channel which comprises:

35 a) transforming a host with a recombinant construct comprising in the 5' to 3' direction a promoter operably linked to the toxic insect ion channel nucleic acid

wherein the promoter comprises a nucleic acid fragment comprising at least one in-frame translational termination codon situated between said promoter and the isolated nucleic acid fragment encoding the toxic insect ion channel, and

further wherein the nucleic acid fragment comprising the translation termination codon is flanked on each end by at least one nucleic acid sequence consisting essentially of excisable sequences; and

b) growing the transformed host under conditions that are suitable for the expression of the recombinant construct.

26. A method for expressing an isolated nucleic acid fragment encoding a toxic insect ion channel which comprises:

a) transforming a host with a recombinant construct comprising in the 5' to 3' direction a promoter operably linked to the toxic insect ion channel nucleic acid

wherein the promoter comprises a nucleic acid fragment consisting essentially of at least one transcription termination nucleic acid fragment and at least one in-frame translational termination codon situated between said promoter and the isolated nucleic acid fragment encoding the toxic insect ion channel, and

further wherein the transcription termination nucleic acid fragment and the translational termination codon are flanked on each end by at least one nucleic acid sequence consisting essentially of excisable sequences; and

b) growing the transformed host under conditions that are suitable for the expression of the recombinant construct.

27. A method for expressing an isolated nucleic acid fragment encoding a toxic insect ion channel which comprises:

a) transforming a host with a recombinant construct comprising in the 5' to 3' direction a promoter operably linked to an isolated nucleic acid fragment encoding the toxic insect ion channel,

wherein said fragment also comprises an intron which interferes with expression of said fragment, and

b) growing the transformed host under conditions that are suitable for the expression of the recombinant construct.

28. The method of any of claims 24, 25 or 26 wherein the excisable sequences are lox sequences.

29. A recombinant construct comprising in the 5' to 3' direction a promoter operably linked to an isolated nucleic acid fragment encoding a toxic insect ion channel

wherein the promoter comprises a transcription termination nucleic acid fragment situated between said promoter and the isolated nucleic acid fragment encoding the toxic insect ion channel, and

further wherein the transcription termination nucleic acid fragment is flanked on each end by at least one nucleic acid sequence consisting essentially of excisable sequences.

30. A recombinant construct comprising in the 5' to 3' direction a promoter operably linked to an isolated nucleic acid fragment encoding a toxic insect ion channel

wherein the promoter comprises a nucleic acid fragment comprising at least one in-frame translational termination codon situated between said promoter and the isolated nucleic acid fragment encoding the toxic insect ion channel, and

further wherein the nucleic acid fragment comprising the translation termination codon is flanked on each end by at least one nucleic acid sequence consisting essentially of excisable sequences.

31. A recombinant construct comprising in the 5' to 3' direction a promoter operably linked to an isolated nucleic acid fragment encoding a toxic insect ion channel

wherein the promoter comprises a nucleic acid fragment consisting essentially of at least one transcription termination nucleic acid fragment and at least one in-frame translational termination codon situated between said promoter and the isolated nucleic acid fragment encoding the toxic insect ion channel, and

further wherein the transcription termination nucleic acid fragment and the translational termination codon are flanked on each end by at least one nucleic acid sequence consisting essentially of excisable sequences.

32. The recombinant construct of any of claim 29, 30 or 31 wherein the excisable sequences are lox sequences.

33. A recombinant construct comprising in the 5' to 3' direction a promoter operably linked to an isolated nucleic acid fragment encoding a toxic insect ion channel

wherein said isolated nucleic acid fragment also comprises an intron which interferes with expression of the toxic insect ion channel.